UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OCT - 6 1994

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT 2,4-DICHLOROPHENOXYACETIC ACID: Chronic Toxicity Study in DOGS.

FROM: Jess Rowland, M.S, Toxicologist 9/27/94
Section II, Toxicology Branch II, Health Effects Division (7509C)

TO: Walter Waldrop / Judy Coombs
Product Manger 71, Reregistration Division

THRU: K. Clark Swentzel, Head 9/30/94
Section II, Toxicology Branch II, Health Effects Division (7509C)
and
Marcia van Gemert, Ph.D., Chief 9/30/94
Toxicology Branch II, Health Effects Division (H7509C)

TASK IDENTIFICATIONS: Submission: S455698 DP Barcode: D197867
PC Code: 030001 Caswell No: 315 MRID No: 430490-01

ACTION REQUESTED: Review a 1-year chronic toxicity study in dogs.

RESPONSE: A Data Evaluation Record is attached and an Executive Summary follows:

EXECUTIVE SUMMARY: In a chronic toxicity study, beagle dogs [5/Sex/Dose] were fed diets containing 2,4-dichlorophenoxyacetic acid at 0, 1, 5, or 7.5 m/kg/day for 52 weeks. Parameters investigated included: mortality; clinical observations; body weights; food consumption; ophthalmology; hematology; clinical chemistry; urinalysis; organ weights; and gross and histopathology. No treatment-related effects were see on survival, clinical signs, ophthalmology, hematology, urinalysis, organ weight or gross pathology at any dose level. Body weight gains of dogs at 1 mg/kg/day were comparable to those of the controls. Body weight gains were impaired in both sexes of dogs at 5 and 7.5 mg/kg/day with the effect being more pronounced in females at the high dose. Alterations seen in serum chemistry parameters in dogs at 5 and 7.5 mg/kg/day were increases in BUN, creatinine and total cholesterol levels and elevations in alanine aminotransferase activity [ALT]. These alterations were corroborated with histopathological changes in the liver and kidneys of these dogs. The increases in BUN and creatinine are compatible with either dehydration or mild renal tubular epithelial compromise while the elevations in ALT activity are indicative of hepatocellular injury. The increases in total cholesterol are nonspecific, but are typically seen with alterations in lipid metabolism by the liver. Histopathology revealed a minimal increase in the frequency and average severity of sinusoidal lining cells of the liver of females only at 5 and 7.5 mg/kg/day and minimal increases in the frequency and average severity of perivascular, chronic active inflammation of the liver and of pigment in the tubular epithelium of the kidneys in both sexes of dogs at 5 and 7.5 mg/kg/day. Under the conditions of this study, the NOEL is 1 mg/kg/day. The LOEL is 5.0 mg/kg/day, based on alterations in serum chemistry with corroborative histopathological lesions in the liver and kidneys.

CORE CLASSIFICATION: Guideline; this study satisfies the requirement (83-1b) for a chronic feeding study in nonrodents.

PRIMARY REVIEWER: Jess Rowland, M.S, Toxicologist 9/29/94
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SECONDARY REVIEWER: K. Clark Swentzel, Head 9/30/94
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DATA EVALUATION RECORD

STUDY TYPE: Chronic Toxicity - Non Rodent. **GUIDELINE:** 83-1(b)

Chemical No. 030001 **MRID No.** 430490-01

TEST MATERIAL: 2,4-Dichlorophenoxyacetic acid (2,4-D)

REGISTRANT: Industry Task Force II on 2,4-D Research Data

TESTING LABORATORY: Hazleton Washington, Inc, Vienna, VA

TITLE: *"52-WEEK DIETARY TOXICITY STUDY WITH 2,4-D IN DOGS"*

STUDY IDENTIFICATION: HWA 2184-124

AUTHOR: Dan W. Dalgard, D.V.M. **REPORT DATE:** December 2, 1993

EXECUTIVE SUMMARY: In a chronic toxicity study, beagle dogs (5/Sex/Dose) were fed diets containing 2,4-dichlorophenoxyacetic acid at 0, 1, 5, or 7.5 mg/kg/day for 52 weeks. Parameters investigated included: mortality; clinical observations; body weights; food consumption; ophthalmoscopic examinations; clinical pathology including hematology, clinical chemistries, and urinalysis; gross pathology; organ weights; and histopathological evaluation. No treatment-related effects were see on survival, clinical signs of toxicity, ophthalmology, hematology, urinalysis, organ weight or gross pathology at any dose level. While body weight gains of dogs at 1 mg/kg/day were comparable to those of the controls, body weight gains were impaired in both sexes of dogs at 5 and 7.5 mg/kg/day with the effect being more pronounced at 7.5 mg/kg/day, especially in females. Alterations seen in serum chemistry parameters in dogs at 5 and 7.5 mg/kg/day were increases in BUN, creatinine and total cholesterol levels and elevations in ALT. These alterations were corroborated with histopathological changes in the liver and kidneys of these dogs. The increases in BUN and creatinine are compatible with either dehydration or mild renal tubular epithelial compromise. The elevations in ALT activity are indicative of hepatocellular injury. The increases in total cholesterol are nonspecific, but are typically seen with alterations in lipid metabolism by the liver. Histopathology revealed a minimal increase in the frequency and average severity of sinusoidal lining cells of the liver of females only at 5 and 7.5 mg/kg/day and minimal increases in the frequency and average severity of perivascular, chronic active inflammation of the liver and of pigment in the tubular epithelium of the kidneys in both sexes of dogs at 5 and 7.5 mg/kg/day. Under the conditions of this study, the NOEL is 1 mg/kg/day; the LOEL of 5.0 mg/kg/day is based on alterations in serum chemistry with histopathological lesions in the liver and kidneys.

CORE CLASSIFICATION: Guideline; this study satisfies the Guideline requirement [§83-1(b)] for a chronic feeding study in nonrodents.

I. INTRODUCTION

This Data Evaluation Report summarizes the experimental procedures and results of a chronic toxicity study in dogs with 2,4-Dichlorophenoxyacetic acid.

II. MATERIALS AND METHODS

1. Test Material

Chemical Name: 2,4-Dichlorophenoxyacetic acid.
Purity: 96.7%
Lot No.: 909
Description: Off white powder.

2. Test Animals

Species: Dogs
Strain: Beagle
Sex: Males and females
Age At Initiation: 4-6 months
Weight At Initiation: 6.5 - 10.1 kg (M) and 6.5 - 8.9 kg,(F)
Identification: A permanent number in the form of a BioMedic, implantable microchip device inserted subcutaneously into the nape of neck.
Acclimation: At least 2 weeks
Health Status: Good
Housing: Individually housed in stainless steel cages.
Food: Purina Certified Canine Diet Meal #5007.
Water: Tap water ad libitum
Environment: Temperature, 66-82°F; Humidity, 20-93%; Light cycle, 12 hr.on/off; Air flow, 10 air changes/hour.

3. Test Material Formulation and Concentration Analysis

The test material was ground into a fine powder using a mortar and pestle. For the purpose of dosage calculations, the test material was assumed to be 100%. The amount of test material necessary for each test group was weighted on an appropriate balance and premixed in a Waring blender with approximately 200 g of feed for approximately 2-3 minutes. The premix was then added to the required amount of feed for each level and mixed at a rate of approximately 1 minute/kg in a Patterson-Kelly twin-shell blender. Test diets were prepared weekly and dietary levels were adjusted weekly in order to achieve target dose levels, based on the most recently recorded body weight and feed consumption values.

Concentration analyses of each dose level was determined weekly for the first four weeks of the study and every fourth week thereafter. Homogeneity analyses was performed once prior to the start of the study and at Week 12. Stability analysis initiated with the start of the study analyzed stability at room temperature and under refrigeration on Day 7 and at freezer-temperature on Day 43.

4. Treatment

Male and female dogs were fed diets containing 2,4-D acid at 1, 5, or 10 mg/kg/day days per week for a period of up to 52 Weeks. Control animals received standard laboratory diet on the same schedule. During study Week 8, the high-dose was decreased from 10 to 7.5 mg/kg/day because of loss of body weight in dogs at this dose.

5. Study Design

Group No.	Treatment	No. of Animals Males	Females	Dose Level mg/kg/day
1	Control	5	5	0
2	Low	5	5	1
3	Mid	5	5	5
4	High	5	5	7.5/10[a]

[a] = During Week 8, this dose was reduced to 7.5 mg/kg/day due to toxicity.

6. Experimental Procedures

Mortality and moribundity checks were performed twice daily. A through weekly physical examination was conducted at each weighing interval. Body weights and food consumption were measured once prior to treatment and weekly thereafter. Ophthalmologic examinations were conducted prior to initiation and at termination. The following hematology and clinical chemistry parameters were measured once prior to treatment and during Weeks 4, 13, 26, 39, and 52; urinalysis tests were performed once prior to initiation and at weeks 26 and 52. The checked (x) parameters were measured.

Hematology

x Hematocrit (HCT)[a]	x Leukocyte count (WBC)[a]
x Hemoglobin (HGB)[a]	x Platelet count[a]
x Erythrocyte count (RBC)[a]	x Leukocyte differential
x Mean corpuscular HGB (MCH)	x Mean corpuscular HGB Concentration (MCHC)
x Mean corpuscular volume (MCV)	x Corrected leukocyte count
x Cell morphology	x Reticulocyte count

Clinical Chemistry

Electrolytes:	Other
x Calcium[a] x Chloride[a] Magnesium[a] x Phosphorus[a] x Potassium[a] x Sodium	x Albumin[a] x Blood Creatinine[a] x Blood Urea Nitrogen[a] x Total Cholesterol[a] x Globulin x Glucose[a] x Total Bilirubin[a] x Total Protein[a] Triglycerides Serum Protein Electrophoresis Triiodothyronine (T_3) Thyroxine (T_4) A/G Ratio
Enzymes:	
x Alkaline phosphatase x Alanine aminotransferase (SGPT)[a] x Aspartate aminotransferase (SGOT)[a] Cholinesterase[b] x Creatinine phosphatase[a] Lactic acid dehydrogenase γ-Glutamyl transpeptidase (GGPT)	

Urinalysis

x Appearance[a]	x Bilirubin[a]
x Specific gravity[a]	x Occult blood[a]
x pH[a]	x Urobilinogen
x Protein[a]	x Glucose[a]
x Ketones[a]	Microscopic examination of sediment[a]

[a] Required for subchronic and chronic studies.
[b] Required only for organophosphates and carbamates.
[c] Required for chronic studies.

7. Termination

After 523 weeks of treatment, all surviving animals were weighed, anesthetized with sodium thiamylal, and exsanguinated. Necropsies were performed on each animal, all gross pathological changes were recorded, and the following organs were weighed.

Adrenals	Brain	Heart	Kidneys	Liver	Pituitary	Thyroid/ parathyroid	Testes	Ovaries

8. Histopathology

The checked (X) tissues from all animals were trimmed and processed for histopathological evaluation.

Digestive System	Respiratory System
x Tongue	x Trachea*
x Salivary gland*	x Lung*
x Esophagus*	Pharynx +
x Stomach	Larynx +
x Duodenum*	Nose +
x Jejunum*	
x Cecum*	Cardiovascular/Hemo.System
x Colon*	x Aorta (thoracic)*
x Rectum*	x Heart*
x Liver*†	x Bone marrow*
x Gall bladder*	x Lymph nodes*
x Pancreas	x Spleen*
	x Thymus*
Neurological System	
x Brain*†	Urinogenital System
x Pituitary*	x Kidneys*‡
Peripheral nerve*#	x Urinary bladder*
x Spinal cord	x Testis*†
(3 levels)*#	x Prostate
x Eyes (optical nerve)*#	x Seminal vesicle
	x Uterus*
Glandular System	x Ovaries*†
x Adrenals*	
x Lacrimal glands#	Others
x Parathyroids*‡	x All gross lesions and masses
x Thyroids*‡	x Skeletal muscle*
x Mammary glands	x Femur (with articulate surface)
	x Sciatic nerve

* Required for subchronic and chronic studies.
+ Required for chronic inhalation study.
# In subchronic studies examined only if indicated by toxicity or target organ involvement.
† Organ weights required in subchronic and chronic studies.
‡ Organ weights required for non-rodent studies.

9. Statistical Analyses

Mean body weights and body weight changes, mean weekly food consumption, total food consumption, clinical pathology data (with the exception of cell morphology and routine urinalysis data), and organ weight data of treated groups were compared statistically against data of the control group of same sex by ANOVA followed by Dunnett's test. All analyses were conducted using two-tailed tests with a minimum significance level of 5%.

10. Regulatory Compliance

A signed statement of No Data Confidentiality Claim was dated 12-2-93. A signed statement dated 12-2-93 indicated that this study was conducted in accordance with the promulgation of the principles of EPA's Good Laboratory Practices [40CR.160]. A signed statement for Potential Adverse Effects, signed and dated 12-2-93 indicated that this study neither meets nor exceeds any of the applicable criteria stipulated in 40 CFR 158.34. A Quality Assurance Statement was signed and dated 11-24-93.

III. RESULTS

Analysis of Diet Mix

Stability analyses of test diet samples indicated 2,4-D to be stable for at least 7 days under refrigeration and at room temperature and for at least 43 days at -20°; percent of target values ranged from 96.2% to 104%,. Homogeneity of analysis within each diet group showed little variation from top, middle and bottom sampling; relative standard deviation values were within 3%. Results of the concentration analyses indicated that all formulations were within ±10% of target. Selected data are tabulated below:

	% of Target					
Week	Males			Females		
mg/kg/day	1	5	7.5	1	5	7.5
1	107	99	100	102	101	101
4	97	98	102	98	103	101
16	101	104	100	95	101	99
28	103	100	110	97	104	95
40	98	100	100	99	101	98
52	104	104	100	104	103	101

1. Survival

Except for one female dog at 5 mg/kg/day that was sacrificed on Day 130 in a moribund condition following a 6-week period of decreased appetite and progressive weight loss, all dogs survived the duration of the study. "Beagle pain syndrome" and/or aseptic meningitis were among conditions considered in the differential diagnosis. Histopathology revealed a non-specific inflammatory condition involving various abdominal organs.

2. Clinical Observations

No treatment-related clinical signs of toxicity were observed. Both sexes of treated as well as control dogs had mucoid, soft or sanguineous feces and other signs frequently seen in this strain/age of dog.

3. Body Weights and Body Weight Changes

Mean body weight data are summarized in Table 1 show that the mean body weights of both sexes of dogs at the high dose [7.5 mg/kg/day] were lower than the respective controls throughout the study with the decrease being more pronounced in females. Females at the high dose showed no appreciable weight gain during the first several weeks of the study. Thereafter, they showed a slow gradual increase but body weights remained well below the controls for the remainder of the study. Because of earlier loss of body weight in dogs at the high dose, the target dose of 10 mg/kg/day was reduced to 7.5 mg/kg/day during Week 8. This change was made following discussion with the toxicologists at the Agency and their concurrence. Mean body weights of both sexes of dogs at 1 or 5 mg/kg/day were comparable to their respective controls.

Table 1. Mean Body Weights (kg) in Dogs Fed 2,4-D for 52 Weeks.

Week	Males				Females			
mg/kg/day	0	1	5	7.5	0	1	5	7.5
1	8.2	9.0	8.5	8.2	7.8	7.6	7.9	7.3
2	8.5	9.1	8.6	8.6	8.0	7.7	8.0	7.5
3	8.9	9.4	8.8	8.6	8.4	7.7	8.2	7.3
4	9.3	9.6	9.1	8.9	8.4	8.0	8.4	7.4
5	9.3	9.9	9.4	8.9	8.6	8.0	8.5	7.9
6	9.7	10.3	9.7	9.1	8.8	8.2	8.7	7.0
7	9.9	10.4	9.8	9.1	8.9	8.3	8.7	7.3
8[a]	10.3	10.7	10.2	9.3	9.1	8.5	9.0	7.5
13	10.9	11.7	10.4	10.2	9.8	9.1	9.3	8.0
19	11.9	12.3	11.1	10.8	10.4	9.1	9.4	8.5
25	12.0	12.2	11.4	10.9	10.6	9.3	9.4	8.5
31	12.5	12.4	11.3	11.1	10.6	9.5	9.8	8.6
37	12.6	12.6	11.6	11.2	10.7	9.6	10.0	8.8
43	12.8	12.7	11.5	11.4	10.9	10.0	9.9	8.7
49	13.0	12.9	11.8	11.5	11.2	10.2	10.0	8.7
53	13.2	12.8	11.8	11.5	11.4	9.9	9.9	8.6

a = Dose level changed from 10 to 7.5 mg/kg/day.

Body weight change data are presented in Tables 2. Although both sexes of dogs at the high dose gained less weight when compared to controls, the decreases reached statistical significance ($p < 0.05$) only in females. Additionally, females at the low- and mid-doses also exhibited statistically significant lower body weight gains during Weeks 1-39.

Table 2. Mean Body Weight Gain (kg) in Dogs Fed 2,4-D for 52 Weeks.

Week	Males				Females			
mg/kg/day	0	1	5	7.5	0	1	5	7.5
1-13	2.7	2.7	1.9	2.0	2.0	1.5	1.4	0.7*
1-26	4.1	3.5	2.9	2.8	2.8	2.0	1.8	1.3*
1-39	4.4	3.7	3.0	3.0	3.1	1.9*	2.0*	1.4*
1-52	4.9	3.8	3.1	3.3	3.3	2.4	2.1	1.2*

* Significantly different from control at $p < 0.05$.

4. Food and Compound Consumption

No statistically significant differences were observed in mean food consumption for either sex at the three dose levels when compared to respective controls. The dose levels achieved for both sexes of dogs at 1 and 5 mg/kg/day were generally quite close to target levels. For the 1 and 5 mg/kg/day dose levels, the mean dose level achieved for Weeks 1-52 were 1.0 and 5.2 for males and 1.0 and 5.0 for females, respectively. More fluctuations were seen at the high-dose, especially during the first several weeks. When the targeted dose was 10 mg/kg/day during Weeks 1-8, the achieved doses were 11.4 and 10.4 for males and females, respectively. Once the target dose was lowered to 7.5 mg/kg/day, the actual dose level was close to the target levels for the duration of the study. Achieved dose during Weeks 9-52 were 8.2 and 7.9 mg/kg/day for males and females, respectively. The overall mean doses achieved at the high dose for the entire study [Weeks 1-52] were 8.2 and 7.9 mg/kg/day for males and females, respectively.

5. Ophthalmology Examination

No treatment-related ocular changes were seen at any dose level.

6. Clinical Pathology

a. Hematology: No treatment-related alterations were seen in any of the hematological parameters. Although various statistically significant findings were noted, they generally were not dose-and/or time-dependent and/or were within laboratory historical control data and were considered to be normal biologic variations.

b. Clinical Chemistry: Treatment-related, statistically significant [$p < 0.05$] increases observed in ALT activity [Table 3] and BUN [Table 4], creatinine [Table 5] and cholesterol [Table 6] concentrations in both sexes of dogs at 5 and 7.5 mg/kg/day. Mean glucose values were significantly decreased at 5 mg/kg/day in males during Weeks 39 and 52 and in females at Week 52, and in both sexes at 7.5 mg/kg/day during Weeks 26, 39 and 52 [Table 7. Differences observed in other clinical chemistry parameters were not dose-and/or time-dependent and/or were within laboratory historical control data and were considered to be normal biologic variations.

Table 3. ALT Levels in Dogs Fed 2,4-D for 52 Weeks.

		ALT [U/L]					
mg/kg/day	Sex	-1	4	13	26	39	52
0.0	M	34	32	38	43	41	43
1.0	M	28	35	41	46	42	46
5.0	M	33	97*	117*	119*	111*	114*
7.5	M	30	176*	116*	152*	107*	138*
0.0	F	30	35	36	33	36	34
1.0	F	28	33	34	34	28	28
5.0	F	28	59	72*	104	72	59
10.0	F	26	79*	115*	109	70	119*

Table 4. BUN Levels in Dogs Fed 2,4-D for 52 Weeks.

		BUN [mg/dL]					
mg/kg/day	Sex	-1	4	13	26	39	52
0.0	M	9	11	12	12	13	12
1.0	M	9	14	16	15	16	15
5.0	M	10	22*	25	26*	28*	26*
7.5	M	8	24*	25*	23*	22*	24*
0.0	F	10	12	14	15	15	13
1.0	F	11	16	16	17	16	16
5.0	F	9	18*	20*	20*	22*	20*
7.5	F	11	30*	27*	28*	27*	30*

Table 5. Creatinine Levels in Dogs Fed 2,4-D for 52 Weeks.

		CREATININE [mg/dL]					
mg/kg/day	Sex	-1	4	13	26	39	52
0.0	M	0.7	0.8	0.8	0.9	0.9	1.0
1.0	M	0.6	0.9	0.9	1.0	1.0	1.0
5.0	M	0.7	1.2*	1.2*	1.4*	1.5*	1.5*
7.5	M	0.7	1.2*	1.2*	1.3*	1.3*	1.4*
0.0	F	0.7	0.9	0.9	1.0	0.9	0.9
1.0	F	0.7	1.0*	0.9	1.0	0.9	0.9
5.0	F	0.7	1.1*	1.2*	1.3*	1.3*	1.5*
7.5	F	0.7	1.4*	1.4*	1.5*	1.5*	1.7*

Table 6. Total Cholesterol Levels in Dogs Fed 2,4-D for 52 Weeks.

		TOTAL CHOLESTEROL [mg/dL]					
mg/kg/day	Sex	-1	4	13	26	39	52
0.0	M	186	158	153	130	126	127
1.0	M	168	169	157	143	133	129
5.0	M	190	201*	205*	194*	175*	174*
7.5	M	191	193	190	171*	164*	167*
0.0	F	154	137	139	159	132	178
1.0	F	184	172*	187	146	133	200
5.0	F	181	189*	192	198	193*	187
7.5	F	167	194	177	182	168	160

Table 7. Glucose Levels in Dogs Fed 2,4-D for 52 Weeks.

		GLUCOSE [mg/dL]					
mg/kg/day	Sex	-1	4	13	26	39	52
0.0	M	114	101	100	104	96	94
1.0	M	118	106	97	101	96	92
5.0	M	112	102	89	94	85*	82*
7.5	M	116	94	91	93*	81*	80*
0.0	F	114	107	98	107	97	94
1.0	F	110	108	99	105	94	97
5.0	F	109	104	91	97	88	81*
7.5	F	111	90*	90	94*	81*	79*

c.Urinalysis: The urinalyses were generally comparable between control and treated groups at all intervals [-1, 26 and 52 Weeks].

8. Organ Weights

Terminal body weights [fasted] were 14% lower than controls for males and 24% [$p < 0.05$] for females at 7.5 mg/kg/day. No treatment-related differences were seen in group mean absolute and relative organ weight values between the treated and control groups.

9. Gross Pathology

No treatment-related gross pathological changes observed at terminal sacrifice.

10. Histopathology

No treatment-related histopathological alterations were seen in either sex at 1.0 mg/kg/day. Histopathological lesions seen in the liver and kidneys of dogs at 5 and 7.5 mg/kg/day are summarized in Table 8. Liver lesions were characterized as a minimal increases in the frequency and average severity of perivascular, chronic active inflammation and sinusoidal lining cell pigment. Kidney lesions consisted of a minimal increase in the frequency and average severity of pigment in the tubular epithelium of the kidneys. Other alterations observed were considered typical findings in clinically normal dogs of this strain and age.

Table 8. Non-neoplastic Lesions in the Liver and Kidneys of Dogs Fed 2,4-D for 52 Weeks.

No. Examined: 8/Sex/Dose	Males				Females			
mg/kg/day	0	1	5	7.5	0	1	5	7.5
Liver: Inflammation, Chronic active, Perivascular	1	1	3	4	0	0	4	3
Pigment, Sinusoidal lining cells	1	0	1	1	1	2	5	4
Kidneys: Pigment, Tubular epithelium	2	4	5	5	1	1	5	5

IV. DISCUSSION

Dietary administration of 2,4-D acid at 1, 5, or 7.5 m/kg/day for 52 weeks did not cause in any adverse effects on survival, clinical signs of toxicity, ophthalmology, hematology, urinalysis, organ weight or gross pathology. Body weight gains were impaired in both sexes at 5 and 7.5 mg/kg/day with the effect being more pronounced at 7.5 mg/kg/day, especially in females where the differences showed statistical significance. The alterations seen in serum chemistry parameters (increases in BUN, creatinine and total cholesterol levels and ALT activity) in dogs at 5 and 7.5 mg/kg/day were corroborated with histopathological changes in the liver and kidneys of these dogs. The increases in BUN and creatinine are compatible with either dehydration or mild renal tubular epithelial compromise. The elevations in ALT activity are indicative of hepatocellular injury. The increases in total cholesterol are nonspecific, but are typically seen with alterations in lipid metabolism by the liver. The decreases in glucose levels in dogs at these doses are attributed to treatment; however, the mechanism is not apparent from the remainder of the clinical pathology data. Histopathology revealed a minimal increase in the frequency and average severity of sinusoidal lining cells of the liver of females only at 5 and 7.5 mg/kg/day and minimal increases in the frequency and average severity of perivascular, chronic active inflammation of the liver and of pigment in the tubular epithelium of the kidneys in both sexes of dogs at 5 and 7.5 mg/kg/day. It is interesting to note that the effects seen in clinical chemistry (i.,e increases in BUN, creatinine, cholesterol and ALT activity) at 5 mg/kg/day in this study were also seen in a 90-day feeding study (MRID No. 427800-01) at a lower dose (3.75 mg/kg/day). Similarly, the liver lesions (perivascular chronic active inflammation) seen in this study at 5 mg/kg/day were also seen in the 90-day study at a higher dose (7.5 mg/kg/day). Based on these findings it is concluded that the dose levels used in this study were adequate to assess the chronic toxicity of 2,4-D in this species.

V. CONCLUSION

Under the conditions of this study, a NOEL of 1 mg/kg/day and a LOEL of 5.0 mg/kg/day is established. The LOEL is based on alterations in serum chemistry with corroborative histopathological lesions in the liver and kidneys.

VI. CORE CLASSIFICATION: Guideline; this study satisfies the Guideline requirement (§ 82-1(b) for a 1-year feeding study in dogs.